Non-covalent weak interactions play many varieties of important roles in biological systems. Recently, we reported the π - π interaction between a coordinated histidine imidazole ring (His81) and the side chains of aromatic amino acids in the second coordination sphere of pseudoazurin (PAz) significantly influences the active site properties¹.

Met16His mutant was designed to know the protonation / deprotonation effect of the newly introduced histidine imidazole on the structure and properties of the blue copper active site. The charge transfer intensity ratio A_{460}/A_{600} is known to reflect the blue copper active structure. The A_{460}/A_{600} of Met16His was 0.5 (pH 7.0), and the redox potential, E° was determined to be 260 mV vs. NHE (pH 7.0). The ratio, A_{460}/A_{600} of Met16His was changed to 0.36 at pH 4.0, and the redox potential was greatly shifted to 400 mV vs. NHE. The EPR and electronic absorption spectra of Met16His mutant suggested the structure of the active site is switched to more axial structure at acidic pH by the protonation of the newly introduced His16 residue.

The CD spectrum of Met16His showed the partially-unfolded CD spectral pattern in contrast to the WT protein at pH 3.5. The ESI-MS of WT and Met16His at pH 3.2 demonstrated the Cu atom elimination from the active site accompanying with the unfolding reaction.

1) Rehab F. Abdelhamid, Yuji Obara, Yoshiko Uchida, Tkamitsu Kohzuma, David M. Dooley, Doreen E. Brown, J. Biol. Inorg. Chem. (2007) 12 : 165-173.

2P046 X-ray crystal structure analysis of a blue copper protein, pseudoazurin at 1.35 Å resolution.

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Pseudoazurin (PAz) is a blue copper protein, which functions as an electron carrier in numerous microorganisms.

The protein contains eight β -sheets giving an overall β -sandwich structure and possesses two extra α -helices at C terminus. The single copper atom is coordinated by the N^{δ} atoms of His40 and His81, the thioether S atom of Met86, and the thiolate S atom of Cys78 and has a distorted tetrahedral geometry. Met16 is located in the vicinity of the Cu^{2+} -coordinated His81 imidazole. Recently, we have reported the π - π interaction between a coordinated His81 imidazole ring and the side chains of newly introduced aromatic amino acids, instead of Met16, in the second coordination sphere of PAz significantly influences the properties of the blue copper site. High resolution crystal structures of PAz and its mutant proteins are required to elucidate the weak interaction effect on the electronic structure of the active site and electron transfer mechanisms.

Hanging drop vapor diffusion method was applied for the crystallization of PAz at 20 °C. Numerous conditions were searched to obtain appropriate crystals for well defined X-ray diffraction data. The newly obtained crystal of PAz is orthorhombic belonging to the space group, $I222$, which is a novel crystal form of PAz. The diffraction data of 1.35 Å resolution were collected at Photon Factory BL17A.

The details of PAz structure and the related spectroscopic properties will be presented and discussed.

2P047 ヘモグロビンの酸素結合のエネルギーランドスケープ描像：カメレオンモデルによる研究

The energy landscape perspective on the oxygen binding of hemoglobin: a study with chameleon model

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Cooperative oxygen binding of hemoglobin is a prototype of the allosteric regulation of proteins. Sigmoidal shape of the oxygen binding curve can be fitted by assuming the existence of T and R quaternary structures in the Monod-Wyman-Changeux (MWC) model, and the atomistic details of this structural change have been described as sequential events starting from oxygen binding to heme and ending with the change of the quaternary structure in the Perutz mechanism. However, recent theoretical studies on protein folding have shown that folding/unfolding transitions are better described as stochastic processes, rather than sequential processes, occurring on the energy landscape, and this "New View" could also be applied to conformational transitions as well. We here present the energy landscape perspective of the conformational transition of hemoglobin by the use of the chameleon model, a derivative of Go model which effectively incorporates the local allosteric communications as the cooperative changes of interaction. Oxygen binding/dissociation are represented implicitly as the stochastic switching on/off of the extra ligand-mediated interactions among the coarse-grained particles surrounding the ligand, allowing us to obtain the concentration-dependent changes of free energy landscape underlying the saturation curve. The stochastic character of the conformational changes, as well as the symmetry and asymmetry among monomers, will be discussed.

2P048 1E1420

タンパク質凝集体に構造多型を生みだすメカニズムとその病理学的役割

Structural polymorphism of protein aggregates and its pathological relevance

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Misfolding of a protein molecule often causes its insoluble aggregation, and formation of inclusion bodies containing protein aggregates is a major pathological change in neurodegenerative disorders. Recent studies have suggested that disease phenotypes are often affected by morphologies of protein aggregates, but it remains obscure in a molecular level how structural polymorphism of protein aggregates is realized.

Among neurodegenerative disorders, more than a hundred mutations in Cu,Zn-superoxide dismutase (SOD1) have been identified to cause amyotrophic lateral sclerosis (ALS). Given that distinct phenotypes are observed with different mutations in SOD1, we have expected a mutation-dependent structure of SOD1 aggregates. First, by utilizing a mass spectrometry, we have identified three regions in an SOD1 sequence, which can be regarded as building blocks in an SOD1 aggregate structure. We have next found that ALS-causing mutations modulate non-native interactions of those three building blocks, leading to the formation of discrete core structures among SOD1 aggregates with different mutations. Notably, a mutation-dependent core structure is found to determine morphological as well as biochemical properties of SOD1 aggregate. Our findings on mutation-dependent structural polymorphism of SOD1 aggregates will, therefore, shed new light not only on the protein chemistry of general aggregation reactions but also on a pathomechanism of neurodegenerative disorders at a molecular level.

Furukawa, Y. et al., J Biol Chem 2010 in press

2P049

MSES 法による sortase A 不規則領域の構造空間探索

Multiscale enhanced sampling simulation for intrinsic disorder protein, sortase A

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In Gram-positive bacteria a transpeptidase, sortase A, cleaves a C-terminal sorting signal of the surface proteins with a conserved LPXTG motif (where X represents any amino acid), leading to adhesion of the C-terminal to the cell wall peptidoglycan. The solution structures of staphylococcus aureus sortase A with and without the LPAT peptide bound reveal that there exists a structurally disordered loop which undergoes a disorder-to-order transition upon peptide binding. Comprehensive conformational sampling of the disordered loop will be needed to elucidate how the flexibility is useful for recognition of the various LPXTG sequences. Here, the free energy landscape will be examined of the sortase disordered loop calculated via the newly developed multiscale enhanced sampling (MSES) simulation.

The MSES simulation enables an efficient and enhanced conformational sampling for arbitrary subspace while retaining a model of the full degree of freedom, i.e., including protein atoms and explicit solvent. Here, the Ca atoms of the disordered loop is used as the degrees of freedom of the subspace. The conformational samplings are performed for both the peptide bound and unbound sortase using the replica exchange MSES simulations and the free energy surfaces calculated. The free energy landscape will be discussed in relation to the position of another flexible loop and the function of the calcium ion bound, which is essential for enzyme activity, yielding the mechanism of the disorder-to-order transition upon peptide binding.

2P050 1E1435

傷害性 T 細胞の機能にヒト主要組織適合複合体の揺らぎが与える影響について

The effect of structural dynamics of the Human Leucocyte Antigen on the function of cytotoxic T Lymphocyte

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Human leucocyte antigens (HLAs), involved in the infection defense system, primarily respond to pathogenic infections on the surface of almost all cells. HLAs present a certain set of peptides on their peptide-binding groove and are recognized by T cell receptors on cytotoxic T lymphocytes (CTLs), resulting in the CTL activation. During the long term non-progression of the HIV infection, two peptides, VY8 (VPLRPMTY) and RY11 (RPQVPLRPMTY) are found dominantly in the peptide-binding groove on HLAs. RY11 has extra 3 residues at the N-terminus. While VY8 is dominant in the early phase of the HIV infection, RY11 in the late phase. The VY8- and RY11-bound HLAs